CHAPTER 2

CHEMICAL METHODS USED IN PURIFICATION

GENERAL REMARKS

Greater selectivity in purification can often be achieved by making use of differences in chemical properties between the substance to be purified and its contaminants. Unwanted metal ions may be removed by precipitation in the presence of a *collector* (see p 49). Sodium borohydride and other metal hydrides transform organic peroxides and carbonyl-containing impurities such as aldehydes and ketones in alcohols and ethers. Many classes of organic chemicals can be purified by conversion into suitable derivatives, followed by regeneration. This chapter describes relevant procedures.

REMOVAL OF TRACES OF METALS FROM REAGENTS

It is necessary to purify the reagents used for determinations of the more common heavy metals. Also, there should be very little if any metallic contamination of many of the materials required for biochemical studies. The main methods for removing impurities of this type are as follows.

Distillation. Reagents such as water, ammonia, hydrochloric acid, nitric acid, perchloric acid (under reduced pressure), and sulphuric acid can be purified in this way using all-glass stills. Isothermal distillation is convenient for ammonia: a beaker containing concentrated ammonia is left alongside a beaker of distilled water for several days in an empty desiccator so that some of the ammonia distils over into the water. Hydrochloric acid can be purified in the same way. The redistilled ammonia should be kept in polyethylene or paraffin-waxed bottles. In some cases, instead of attempting to purify a salt it is simpler to synthesise it from distilled components. Ammonium acetate is an example.

Use of ion-exchange resin. Application of ion-exchange columns has greatly facilitated the removal of heavy metal ions such as Cu^{2+}, Zn^{2+} and Pb^{2+} from aqueous solutions of many reagents. Thus, sodium salts and sodium hydroxide can be purified by passage through a column of a cation-exchange resin in its sodium form. Similarly, for acids, a resin in its H^+ form is used. In some cases, where metals form anionic complexes, they can be removed by passage through an anion-exchange resin. Iron in hydrochloric acid solution is an example.
Ion exchange resins are also useful for demineralising biochemical preparations such as proteins. Removal of metal ions from protein solutions using polystyrene-based resins, however, may lead to protein denaturation. This difficulty may be avoided by using a weakly acidic cation exchanger such as Bio-Rex 70 (which is a carboxylic acid exchange resin based on a polyacrylic lattice).
Heavy metal contamination of pH buffers can be removed by passage of the solutions through a Chelex X-100 column. For example when a solution of 0.02M HEPES containing 0.2M KCl (1L, pH 7.5) alone or with calmodulin, is passed through a column of Chelex X-100 (60g) in the K^+ form the level of Ca^{2+} ions falls to less than 2×10^{-7} M as shown by atomic absorption spectroscopy. Such solutions should be stored in polyethylene containers that have been washed with boiling deionised water (5min) and rinced several times with deionised water. TES and Tris have been similarly decontaminated from metal ions (see reference on atomic absorption analysis on p 62).
Water, with very low concentrations of ionic impurities (and approaching conductivity standards), is very readily obtained by percolation through alternate columns of cation- and anion-exchange resins, or through a mixed-bed resin, and many commercial devices are available for this purpose. For some applications, this method is unsatisfactory because the final water may contain traces of organic material after passage through the columns. However, organic matter can also be removed by using yet another special column in series for this purpose (see Milli Q water preparation, Millipore Corpn).

Precipitation. In removing traces of impurities by precipitation it is necessary to include a material to act as a *collector* of the precipitated substance so as to facilitate its removal by filtration or decantation. Aqueous hydrofluoric acid can be freed from lead by adding 1ml of 10% strontium chloride per 100ml of acid, lead being co-precipitated as lead fluoride with the strontium fluoride. If the acid is decanted from the precipitate and the process repeated, the final lead content in the acid is less than 0.003 ppm. Similarly, lead can be precipitated from a nearly saturated sodium carbonate solution by adding 10% strontium chloride dropwise (1-2ml per 100ml), then filtering. (If the sodium carbonate is required as a solid, the solution can be evaporated to dryness in a platinum dish.) Removal of lead from potassium chloride uses precipitation as lead sulphide, followed, after filtration, by evaporation and recrystallisation of the potassium chloride.

Several precipitation methods are available for iron. It has been removed from potassium thiocyanate solutions by adding a few milligrams of an aluminium salt, then precipitating aluminum and iron as their hydroxides by adding a few drops of ammonia. Iron is also carried down on the hydrated manganese dioxide precipitate formed in cadmium chloride or cadmium sulphate solutions by adding 0.5% aqueous potassium permanganate (0.5ml per 100ml of solution), sufficient ammonia to give a slight precipitate, and 1ml of ethanol. The solution is heated to boiling to coagulate the precipitate, then filtered. For the removal of iron from sodium potassium tartrate, a small amount of cadmium chloride solution and a slight excess of ammonium sulphide are added, the solution is stood for 1 hour, and the sulphide precipitate is filtered off. Ferrous iron can be removed from copper solutions by adding some hydrogen peroxide to the solution to oxidise the iron, followed by precipitation of ferric hydroxide by adding a small amount of sodium hydroxide.

Traces of calcium can be removed from solutions of sodium salts by precipitation at pH 9.5-10 as its 8-hydroxyquinolinate. The excess of 8-hydroxyquinoline acts as a *collector*. The magnesium content of calcium chloride solutions can be reduced by making them about 0.1M in sodium hydroxide and filtering.

Extraction. In some cases, a simple solvent extraction is sufficient to remove a particular impurity. For example, traces of gallium can be removed from titanous chloride in hydrochloric acid by extraction with isopropyl ether. Similarly, ferric chloride can be removed from aluminium chloride solutions containing hydrochloric acid by extraction with ethyl ether. Usually, however, it is necessary to extract with an organic solvent in the presence of a suitable complexing agent such as dithizone or sodium diethyl dithiocarbamate. When the former is used, weakly alkaline solutions are extracted with dithizone in chloroform (at about 25mg/L of chloroform) or carbon tetrachloride until the colour of some fresh dithizone solution remains unchanged after shaking. Excess dithizone is taken out by extracting with the pure solvent, the last traces of which, in turn, are removed by aeration. This method has been used with aqueous solutions of ammonium hydrogen citrate, potassium bromide, potassium cyanide, sodium acetate and sodium citrate. The advantage of dithizone for such a purpose lies in the wide range of metals with which it combines under these conditions. 8-Hydroxyquinoline (oxine) can also be used in this way. Sodium diethyl dithiocarbamate has been used to purify aqueous hydroxylamine hydrochloride (made just alkaline to thymol blue by adding ammonia) from copper and other heavy metals by repeated extraction with chloroform until no more diethyl dithiocarbamate remained in the solution (which was then acidified to thymol blue by adding hydrochloric acid).

Complexation. Although not strictly a removal of an impurity, addition of a suitable complexing agent such as ethylenediaminetetra-acetic acid often overcomes the undesirable effects of contaminating metal ions by reducing the concentrations of the free metal species to very low levels. For a detailed discussion of this *masking*, see *Masking and Demasking of Chemical Reactions*, D.D.Perrin, Wiley-Interscience, New York, 1970.

USE OF METAL HYDRIDES

This group of reagents has become commercially available in large quantities; some of its members - notably lithium aluminium hydride ($LiAlH_4$), calcium hydride (CaH_2), sodium borohydride ($NaBH_4$) and potassium borohydride (KBH_4) - have found widespread use in the purification of chemicals.

Lithium aluminium hydride. This solid is stable at room temperature, and is soluble in ether-type solvents. It reacts violently with water, liberating hydrogen, and is a powerful drying and reducing agent for organic compounds. It reduces aldehydes, ketones, esters, carboxylic acids, peroxides, acid anhydrides and acid chlorides to the corresponding alcohols. Similarly, amides, nitriles, aldimines and aliphatic nitro compounds yield amines, while aromatic nitro compounds are converted to azo compounds. For this reason it finds extensive application in purifying organic chemical substances by the removal of water and carbonyl containing impurities as well as peroxides formed by autoxidation. Reactions can generally be carried out at room temperature, or in refluxing ethyl ether, at atmospheric pressure. *When drying organic liquids with this reagent it is important that the concentration of water in the liquid is below 0.1% otherwise a violent reaction or* **EXPLOSION** *may occur. The mixing of the liquid with the reagent should be performed at ice bath temperature and under a reflux condenser.*

Calcium hydride. This powerful drying agent is suitable for use with hydrogen, argon, helium, nitrogen, hydrocarbons, chlorinated hydrocarbons, esters and higher alcohols.

Sodium borohydride. This solid which is stable in dry air up to 300° like potassium borohydride, is a less powerful reducing agent than lithium aluminium hydride, from which it differs also by being soluble in hydroxylic solvents and to a lesser extent in ether-type solvents. Sodium borohydride forms a dihydrate melting at 36-37°, and its aqueous solutions decompose slowly unless stabilised to above pH 9 by alkali. (For example, a useful solution is one nearly saturated at 30-40° and containing 0.2% sodium hydroxide.) Its solubility in water is 25, 55 and 88g per 100ml of water at 0°, 25° and 60°, respectively. Its aqueous solutions are rapidly decomposed by boiling or acidification. The reagent, available either as a hygroscopic solid or as an aqueous sodium hydroxide solution, is useful as a water soluble reducing agent for aldehydes, ketones and organic peroxides. This explains its use for the removal of carbonyl-containing impurities and peroxides from alcohols, polyols, esters, polyesters, amino-alcohols, olefines, chlorinated hydrocarbons, ethers, polyethers, amines (including aniline), polyamines and aliphatic sulphonates.
Purifications can be carried out conveniently using alkaline aqueous or methanolic solutions, allowing the reaction mixture to stand at room temperature for several hours. Other solvents that can be used with this reagent include isopropyl alcohol (without alkali), amines (including liquid ammonia, in which its solubility is 104g per 100g of ammonia at 25°, and ethylenediamine), diglyme, formamide, dimethylformamide and tetrahydrofurfuryl alcohol. Alternatively, the material to be purified can be percolated through a column of the borohydride. In the absence of water, sodium borohydride solutions in organic solvents such as dioxane or amines decompose only very slowly at room temperature. Treatment of ethers with sodium borohydride appears to inhibit peroxide formation.

Potassium borohydride. Potassium borohydride is similar in properties and reactions to sodium borohydride, and, like it, is used as a reducing agent for removing aldehydes, ketones and organic peroxides. It is non-hygroscopic and can be used in water, ethanol, methanol or water-alcohol mixtures, provided some alkali is added to minimise decomposition, but it is somewhat less soluble than sodium borohydride in most solvents. For example, its solubility in water at 25° is 19g per 100ml of water (compare sodium borohydride, 55g).

PURIFICATION *via* DERIVATIVES

Relatively few derivatives of organic substances are suitable for use as aids to purification. This is because of the difficuly in regenerating the starting material. For this reason, we list below, the common methods of preparation of derivatives that can be used in this way.
Whether or not any of these derivatives is likely to be satisfactory for the use of any particular case will depend on the degree of difference in properties, such as solubility, volatility or melting point, between the starting material, its derivative and likely impurities, as well as on the ease with which the substance can be recovered. Purification *via* a derivative is likely to be of most use when the quantity of pure material that is required is not too large. Where large quantities (for example, more than 50g) are available, it is usually more economical to purify the material directly and discard larger fractions (for example, in distillations and recrystallisations).

The most generally useful purifications *via* derivatives are as follows:

Alcohols. Aliphatic or aromatic alcohols are converted to solid esters. *p*-Nitrobenzoates are the most convenient esters to form because of their sharp melting points, and the ease with which they can be recrystallised and the alcohol recovered. The *p*-nitrobenzoyl chloride used in the esterification is prepared by refluxing dry *p*-nitrobenzoic acid with a 3 molar excess of thionyl chloride for 30min on a steam bath (*in a fume cupboard*). The solution is cooled slightly and the excess thionyl chloride is distilled off under (water-pump) vacuum, keeping the temperature below 40°. Dry toluene is added to the residue in the flask, then distilled off under vacuum, the process being repeated two or three times to ensure complete removal of thionyl chloride, hydrogen chloride and sulphur dioxide. (This freshly prepared *p*-nitrobenzoyl chloride cannot be stored without decomposition; it should be used directly.) A solution of the acid chloride (1mol) in dry toluene or alcohol-free chloroform (distilled from P_2O_5 or by passage through an activated Al_2O_3 column) under a reflux condenser is cooled in an ice bath while the alcohol (1mol), with or without a solvent (preferably miscible with toluene or alcohol-free chloroform), is added dropwise to it. When addition is over and the reaction subsides, the mixture is refluxed for 30min and the solvent is removed under reduced pressure. The solid ester is then recrystallised to constant melting point from toluene, acetone, light petroleum or mixtures of these, but not from alcohols.
Hydrolysis of the ester is achieved by refluxing in aqueous N or 2N NaOH solution until the insoluble ester dissolves. The solution is then cooled, and the alcohol is extracted into a suitable solvent, e.g. ether, toluene or alcohol-free chloroform. The extract is dried ($CaSO_4$, $MgSO_4$) and distilled, then fractionally distilled if liquid or recrystallised if solid. (The nitro acid can be recovered by acidification of the aqueous layer.) In most cases where the alcohol to be purified is readily freed from ethanol, the hydrolysis of the ester is best achieved with N or 2N ethanolic NaOH or 85% aqueous ethanolic N NaOH. The former is prepared by dissolving the necessary alkali in a minimum volume of water and diluting with absolute alcohol. The ethanolic solution is refluxed for one to two hours and hydrolysis is complete when an aliquot gives a clear solution on dilution with four or five times its volume of water. The bulk of the ethanol is

distilled off and the residue is extracted as above. Alternatively, use can be made of ester formation with benzoic acid, toluic acid or 3,5-dinitrobenzoic acid, by the above method.
Other derivtives can be prepared by reaction of the alcohol with an acid anhydride. For example, phthalic or 3-nitrophthalic anhydride (1 mol) and the alcohol (1mol) are refluxed for half to one hour in a non-hydroxylic solvent, e.g. toluene or alcohol-free chloroform, and then cooled. The phthalate ester crystallises out, is precipitated by the addition of light petroleum or is isolated by evaporation of the solvent. It is recrystallised from water, 50% aqueous ethanol, toluene or light petroleum. Such an estér has a characteristic melting point and the alcohol can be recovered by acid or alkaline hydrolysis.

Aldehydes and Ketones. The best derivative from which an aldehyde can be recovered readily is its bisulphite addition compound, the main disadvantage being the lack of a sharp melting point. The aldehyde (sometimes in ethanol) is shaken with a cold saturated solution of sodium bisulphite until no more solid adduct separates. The adduct is filtered off, washed with a little water, then alcohol. A better reagent is freshly prepared saturated aqueous sodium bisulphite solution to which 75% ethanol is added to near-saturation. (Water may have to be added dropwise to render this solution clear.) With this reagent the aldehyde need not be dissolved separately in alcohol and the adduct is finally washed with alcohol. The aldehyde is recovered by dissolving the adduct in the least volume of water and adding an equivalent quantity of sodium carbonate (not sodium hydroxide) or concentrated hydrochloric acid to react with the bisulphite, followed by steam distillation or solvent extraction.
Other derivatives that can be prepared are the Schiff bases and semicarbazones. Condensation of the aldehyde with an equivalent of primary aromatic amine yields the Schiff base, for example aniline at 100° for 10-30min.
Semicarbazones are prepared by dissolving semicarbazide hydrochloride (*ca* 1g) and sodium acetate (*ca* 1.5g) in water (8-10ml) and adding the aldehyde or ketone (0.5-1g) and stirring. The semicarbazone crystallises out and is recrystallised from ethanol or aqueous ethanol. These are hydrolysed by steam distillation in the presence of oxalic acid or better by exchange with pyruvic acid (Hershberg *JOC* **13** 542 *1948*).

Amines. (a) Picrates: The most versatile derivative from which the free base can be readily recovered is the picrate. This is very satisfactory for primary and secondary aliphatic amines and aromatic amines and is particularly so for heterocyclic bases. The amine, dissolved in water, alcohol or benzene, is treated with excess of a saturated solution of picric acid in water, alcohol or benzene, respectively, until separation of the picrate is complete. If separation does not occur, the solution is stirred vigorously and warmed for a few minutes, or diluted with a solvent in which the picrate is insoluble. Thus, a solution of the amine and picric acid in ethanol or benzene can be treated with benzene or light petroleum, repectively, to precipitate the picrate. Alternatively, the amine can be dissolved in alcohol and aqueous picric acid added. The picrate is filtered off, washed with water, ethanol or benzene, and recrystallised from boiling water, ethanol, methanol, aqueous ethanol or methanol, chloroform or benzene. The solubility of picric acid in water, ethanol and benzene is 1.4, 6.23 and 5.27% respectively at 20°.
It is not advisable to store large quantities of picrates for long periods, *particularly when they are dry due to their potential* **EXPLOSIVE** *nature.* The free base should be recovered as soon as possible. The picrate is suspended in an excess of 2N aqueous NaOH and warmed a little. Because of the limited solubility of sodium picrate, excess hot water must be added. Alternatively, because of the greater solubility of lithium picrate, aqueous 10% lithium hydroxide solution can be used. The solution is cooled, the amine is extracted with a suitable solvent such as ethyl ether or toluene, washed with 5N NaOH until the alkaline solution remains colourless, then with water, and the extract is dried with anhydrous sodium carbonate. The solvent is distilled off and the amine is fractionally distilled (under reduced pressure if necessary) or recrystallised.
If the amines are required as their hydrochlorides, picrates can often be decomposed by suspending them in much acetone and adding two equivalents of 10N HCl. The hydrochloride of the base is filtered off, leaving the picric acid in the acetone. Dowex No 1 anion-exchange resin in the chloride form is useful for changing solutions of the more soluble picrates (for example, of adenosine) into solutions of their hydrochlorides, from which sodium hydroxide precipitates the free base .

(b) Salts: Amines can also be purified *via* their salts, e.g. hydrochlorides. A solution of the amine in dry toluene, ether, methylene chloride or chloroform is saturated with dry hydrogen chloride (generated by addition of concentrated sulphuric acid to dry sodium chloride, or to concentrated HCl followed by drying the gas through sulphuric acid, or from a hydrogen chloride cylinder) and the insoluble hydrochloride is filtered off and dissolved in water. The solution is made alkaline and the amine is extracted, as above. Hydrochlorides can also be prepared by dissolving the amine in ethanolic HCl and adding ether or light petroleum. Where hydrochlorides are too hygroscopic or too soluble for satisfactory isolation, other salts, e.g. nitrate, sulphate, bisulphate or oxalate, can be used.

(c) Double Salts: The amine (1mol) is added to a solution of anhydrous zinc chloride (1mol) in concentrated hydrochloric acid (42ml) in ethanol (200ml, or less depending on the solubility of the double salt). The solution is stirred for 1h and the precipitated salt is filtered off and recrystallised from ethanol. The free base is recovered by adding excess of 5-10N NaOH (to dissolve the zinc hydroxide that separates) and is steam distilled. Mercuric chloride in hot water can be used instead of zinc chloride and the salt is crystallised from 1% hydrochloric acid. Other double salts have been used, e.g. cuprous salts, but are not as convenient as the above salts.

(d) N-Acetyl derivatives: Purification as their N-acetyl derivatives is satisfactory for primary, and to a limited extent secondary, amines. The base is refluxed with slightly more than one equivalent of acetic anhydride for half to one hour, cooled and poured into ice-cold water. The insoluble derivative is filtered off, dried, and recrystallised from water, ethanol, aqueous ethanol, benzene or benzene-light petroleum. The derivative is then hydrolysed by

refluxing with 70% sulphuric acid for a half to one hour. The solution is cooled, poured onto ice, and made alkaline. The amine is steam distilled or extracted as above. Alkaline hydrolysis is very slow.

(e) N-Tosyl derivatives: Primary and secondary amines are converted into their tosyl derivatives by mixing equimolar amounts of amine and toluene-*p*-sulphonyl chloride in dry pyridine (*ca* 5-10mols) and allowing to stand at room temperature overnight. The solution is poured into ice-water and the pH adjusted to 2 with HCl. The solid derivative is filtered off, washed with water, dried (vac. desiccator) and recrystallised from an alcohol or aqueous alcohol solution to a sharp melting point. The derivative is decomposed by dissolving in liquid ammonia (*fume cupboard*) and adding sodium metal (in small pieces with stirring) until the blue colour persists for 10-15min. Ammonia is allowed to evaporate (*fume cupboard*), the residue treated with water and the solution checked that the pH is above 10. If the pH is below 10 then the solution has to be basified with 2N NaOH. The mixture is extracted with ether or toluene, the extract is dried (K_2CO_3), evaporated and the residual amine recrystallised if solid or distilled if liquid.

Aromatic hydrocarbons. (a) Adducts: Aromatic hydrocarbons can be purified as their picrates using the procedures described for amines. Instead of picric acid, 1,3,5-trinitrobenzene or 2,4,7-trinitrofluorenone can also be used. In all these cases, following recrystallisation, the hydrocarbon can be isolated either as described for amines or by passing a solution of the adduct through an activated alumina column and eluting with toluene or light petroleum. The picric acid and nitro compounds are more strongly adsorbed on the column.

(b) Sulphonation: Naphthalene, xylenes and alkyl benzene can be purified by sulphonation with concentrated sulphuric acid and crystallisation of the sodium sulphonates. The hydrocarbon is distilled out of the mixture with superheated steam.

Carboxylic acids (a) 4-Bromophenacyl esters: A solution of the sodium salt of the acid is prepared. If the salt is not available, the acid is dissolved in an equivalent of aqueous NaOH and the pH adjusted to 8-9 with this base. A solution of one equivalent of 4-bromophenacyl bromide (for a monobasic acid, two equivalents for a dibasic acid, etc) in ten times its volume of ethanol is then added. The mixture is heated to boiling, and, if necessary, enough ethanol is added to clarify the solution which is then refluxed for half to three hours depending on the number of carboxylic groups that have to be esterified. (One hour is generally sufficient for monocarboxylic acids.) On cooling, the ester should crystallise out. If it does not do so, the solution is heated to boiling, and enough water is added to produce a slight turbidity. The solution is again cooled. The ester is collected, and recrystallised or fractionally distilled.
The ester is hydrolysed by refluxing for 1-2h with 1-5% of barium carbonate suspended in water or with aqueous sodium carbonate solution. The solution is cooled and extracted with ether, toluene or chloroform. It is then acidified and the acid is collected by filtration or extraction, and recrystallised or fractionally distilled.
p-Nitrobenzyl esters can be prepared in an analogous manner using the sodium salt of the acid and *p*-nitrobenzyl bromide. They are readily hydrolysed.

(b) Alkyl esters: Of the alkyl esters, methyl esters are the most useful because of their rapid hydrolysis. The acid is refluxed with one or two equivalents of methanol in excess alcohol-free chloroform (or methylene chloride) containing about 0.1g of toluene-*p*-sulphonic acid (as catalyst), using a Dean and Stark trap. (The water formed by the esterification is carried away into the trap.) When the theoretical amount of water is collected in the trap, esterification is complete. The chloroform solution in the flask is washed with 5% aqueous sodium carbonate solution, then water, and dried over sodium sulphate or magnesium sulphate. The chloroform is distilled off and the ester is fractionally distilled through an efficient column. The ester is hydrolysed by refluxing with 5-10% aqueous NaOH solution until the insoluble ester has completely dissolved. The aqueous solution is concentrated a little by distillation to remove all of the methanol. It is then cooled and acidified. The acid is either extracted with ether, toluene or chloroform, or filtered off and isolated as above. Other methods for preparing esters are available.

(c) Salts: The most useful salt derivatives for carboxylic acids are the isothiouronium salts. These are prepared by mixing almost saturated solutions containing the acid (carefully neutralised with N NaOH using phenolphthalein indicator) then adding two drops of N HCl and an equimolar amount of *S*-benzylisothiouronium chloride in ethanol and filtering off the salt that crystallises out. After recrystallisation from water, alcohol or aqueous alcohol the salt is decomposed by suspending or dissolving in 2N HCl and extracting the carboxylic acid in ether, chloroform or toluene.

Hydroperoxides. These can be converted to their sodium salts by precipitation below 30° with aqueous 25% NaOH. The salt is then decomposed by addition of solid (powdered) carbon dioxide and extracted with low-boiling petroleum ether. The solvent should be removed under reduced pressure below 20°. **The apparatus should be adequately shielded at all times for the safety of the operator from EXPLOSIONS.**

Ketones. (a) Bisulphite adduct: The adduct can be prepared and decomposed as described for aldehydes. Alternatively, because no Cannizzaro reaction is possible, it can also be decomposed with 0.5N NaOH.

(b) Semicarbazones: A powdered mixture of semicarbazide hydrochloride (1mol) and anhydrous sodium acetate (1.3mol) is dissolved in water by gentle warming. A solution of the ketone (1mol) in the least volume of ethanol needed to dissolve it is then added. The mixture is warmed on a water bath until separation of the semicarbazone is complete. The solution is cooled, and the solid is filtered off. After washing with a little ethanol followed by water, it is recrystallised from ethanol or dilute aqueous ethanol. The derivative should have a characteristic melting point. The semicarbazone is decomposed by refluxing with excess of oxalic acid or with aqueous sodium carbonate solution. The ketone (which steam distils) is distilled off. It is extracted or separated from the

distillate (after saturating with NaCl), dried with $CaSO_4$ or $MgSO_4$ and fractionally distilled using an efficient column (under vacuum if necessary).

Phenols. The most satisfactory derivatives for phenols that are of low molecular weight or monohydric are the benzoate esters. (Their acetate esters are generally liquids or low-melting solids.) Acetates are more useful for high molecular weight and polyhydric phenols.

(a) Benzoates: The phenol (1mol) in 5% aqueous NaOH is treated (while cooling) with benzoyl chloride (1mol) and the mixture is stirred in an ice bath until separation of the solid benzoyl derivative is complete. The derivative is filtered off, washed with alkali, then water, and dried (in a vacuum desiccator over NaOH). It is recrystallised from ethanol or dilute aqueous ethanol. The benzoylation can also be carried out in dry pyridine at low temperature (*ca* 0°) instead of in NaOH solution, finally pouring the mixture into water and collecting the solid above. The ester is hydrolysed by refluxing in an alcohol (for example, ethanol, *n*-butanol) containing two or three equivalents of the alkoxide of the corresponding alcohol (for example sodium ethoxide or sodium *n*-butoxide) and a few (*ca* 5-10) millilitres of water, for half to three hours. When hydrolysis is complete, an aliquot will remain clear on dilution with four to five times its volume of water. Most of the solvent is distilled off. The residue is diluted with cold water and acidified, and the phenol is steam distilled. The latter is collected from the distillate, dried and either fractionally distilled or recrystallised.

(b) Acetates: These can be prepared as for the benzoates using either acetic anhydride with 3N NaOH or acetyl chloride in pyridine. They are hydrolysed as described for the benzoates. This hydrolysis can also be carried out with aqueous 10% NaOH solution, completion of hydrolysis being indicated by the complete dissolution of the acetate in the aqueous alkaline solution. On steam distillation, acetic acid also distils off but in these cases the phenols (see above) are invariably solids which can be filtered off and recrystallised.

Phosphate and phosphonate esters. These can be converted to their nitrate addition compounds. The crude or partially purified ester is saturated with uranyl nitrate solution and the adduct filtered off. It is recrystallised from *n*-hexane, toluene or ethanol. For the more soluble members crystallisation from hexane using low temperatures (-40°) has been successful. The adduct is decomposed by shaking with sodium carbonate solution and water, the solvent is steam distilled (if hexane or toluene is used) and the ester is collected by filtration. Alternatively, after decomposition, the organic layer is separated, dried with $CaCl_2$ or BaO, filtered, and fractionally distilled at high vacuum.

Alternatively, impurities can sometimes be removed by conversion to derivatives under conditions where the major component does not react. For example, normal (straight-chain) paraffins can be freed from unsaturated and branched-chain components by taking advantage of the greater reactivity of the latter with chlorosulphonic acid or bromine. Similarly, the preferential nitration of aromatic hydrocarbons can be used to remove e.g. benzene or toluene from cyclohexane by shaking for some hours with a mixture of concentrated nitric acid (25%), sulphuric acid (58%), and water (17%).

GENERAL METHODS FOR THE PURIFICATION OF CLASSES OF COMPOUNDS

Chapters 3, 4 and 5 list a large number of individual compounds, with a brief statement of how each one may be purified. For substances that are not included in these chapters the following procedures may prove helpful.

If the laboratory worker does not know of a reference to the preparation of a commercially available substance, he may be able to make a reasonable guess at the synthetic method used from published laboratory syntheses. This information, in turn, can simplify the necessary purification steps by suggesting probable contaminants. However, for other than macromolecules it is important that at least the NMR and IR spectra of the substance be measured. These measurements require no more than two to three milligrams (which are recoverable) of material and provides a considerable amount of information about the substance. Three volumes on the NMR spectra [C.J.Pouchert and J.Behnke, *The Aldrich Library of ^{13}C and ^{1}H FT-NMR Spectra, Vols I—3,* Aldrich Chemical Co., Inc, Milwaukee, WI, *1993*], and one on the infrared spectra [C.J.Pouchert, *The Aldrich Library of FT-IR Spectra*, 3nd ed, Aldrich Chemical Co., Milwaukee, WI, *1989*], as well as computer software [*FT-IR Peak-search Data Base and Software*, for Apple IIE, IIC and II Plus computers; and for IBM PC computers, Nicholet Instruments, Madison, WI, 1984] contain data for all the compounds in the Aldrich catalogue and are extremely useful for identifying compounds and impurities. If the material appears to have several impurities these spectra should be followed by examination of their chromatographic properties and spot tests. Purification methods can then be devised to remove these impurities, and a monitoring method will have already been established.

Physical methods of purification depend largely on the melting and boiling points of the materials.. For gases and low-boiling liquids use is commonly made of the *freeze-pump-thaw* (see p. 19) procedure. Gas chromatography is also useful, especially for low-boiling point liquids. Liquids are usually purified by refluxing with drying agents, acids or bases, reducing agents, charcoal, etc., followed by fractional distillation under reduced pressure. For solids, general methods include fractional freezing of the melted material, taking the middle fraction. A related procedure is zone refining. Another procedure is sublimation of the solid under reduced pressure. The other commonly used method for purifying solids is by recrystallisation from a solution in a suitable solvent, by cooling with or without the prior addition of a solvent in which the solute is not very soluble.

Purification becomes meaningful only insofar as adequate tests of purity are applied: the higher the degree of purity that is sought, the more stringent must these tests be. If the material is an organic solid, its melting point should first be taken and compared with the recorded value. Also, as part of this preliminary examination, the sample might be examined by thin layer (or paper) chromatography (see E.Demole, *Chromatographic Reviews*, **4** 26 *1962*) in several different solvent systems and in high enough concentrations to facilitate the detection of minor components. On the other hand, if the substance is a liquid, its boiling point should be measured. If, further, it is a high boiling liquid, its chromatographic behaviour should be examined. Liquids, especially volatile ones, can be studied very satisfactorily by gas chromatography, preferably using at least two different stationary phases.

Application of these tests at successive steps will give a good indication of whether or not the purification is satisfactory and will also show when adequate purification has been achieved.

The nature of the procedure will depend to a large extent on the quantity of purified material that is required. For example, for small quantities (50-250mg) of a pure liquid, preparative gas chromatography is probably the best method. Two passes through a suitable column may well be sufficient. Similarly, for small amounts (100-500mg) of an organic solid, column chromatography is likely to be very satisfactory., the eluate being collected as a number of separate fractions (*ca* 5-10ml) which are examined by FT-IR, NMR or UV spectroscopy, TLC or by some other appropriate analytical technique. (For information on suitable adsorbents and eluents the texts referred to in the bibliography at the end of Chapters 1 and 2 should be consulted.) Preparative thin layer chromatography or HPLC can be used successfully for purifying up to 500mg of solid.

Where larger quantities (upwards of 1g) are required, most of the impurities should be removed by preliminary treatments, such as solvent extraction, liquid-liquid partition, or conversion to a derivative (*vide supra*) which can be purified by crystallisation or fractional distillation before being reconverted to the starting material. The substance is then crystallised or distilled. If the final amounts must be in excess of 25g, preparation of a derivative is sometimes omitted because of the cost involved. In all of the above cases, purification is likely to be more laborious if the impurity is an isomer or a derivative with closely similar physical properties.

In the general methods of purification described below, it is assumed that the impurities belong essentially to a class of compounds different from the one being purified. They are suggested for use in cases where substances are not listed in Chapters 3, 4 and the low molecular weight compounds in Chapter 5. In such cases, the experimenter is advised to employ them in conjunction with information given in these chapters for the purification of suitable analogues. Also, for a wider range of drying agents, solvents for extraction and solvents for recrystallisation, the reader is referred to Chapter 1. See Chapter 5 for general purification procedures used for macromolecules.

GENERAL PROCEDURES FOR THE PURIFICATION OF SOME CLASSES OF ORGANIC COMPOUNDS

Acetals. These are generally diethyl or dimethyl acetal derivatives of aldehydes. They are more stable to alkali than to acids. Their common impurities are the corresponding alcohol, aldehyde and water. Drying with sodium wire removes alcohols and water, and polymerizes aldehydes so that, after decantation, the acetal can be fractionally distilled. In cases where the use of sodium is too drastic, aldehydes can be removed by shaking with alkaline hydrogen peroxide solution and the acetal is dried with sodium carbonate or potassium carbonate. Residual water and alcohols (up to *n*-propyl) can be removed with Linde type 4A molecular sieves. The acetal is then filtered and fractionally

distilled. Solid acetals (i.e. acetals of high molecular weight aldehydes) are generally low-melting and can be recrystallised from low-boiling petroleum ether, toluene or a mixture of both.

Acids. (a) Carboxylic: Liquid carboxylic acids are first freed from neutral and basic impurities by dissolving them in aqueous alkali and extracting with ethyl ether. (The pH of the solution should be at least three units above the pK_a of the acid). The aqueous phase is then acidified to a pH at least three units below the pK_a of the acid and again extracted with ether. The extract is dried with magnesium sulphate or sodium sulphate and the ether is distilled off. The acid is fractionally distilled through an efficient column. It can be further purified by conversion to its methyl or ethyl ester (see p. 52) which is then fractionally distilled. Hydrolysis yields the original acid which is again purified as above.

Acids that are solids can be purified in this way, except that distillation is replaced by repeated crystallisation (preferable from at least two different solvents such as water, alcohol or aqueous alcohol, toluene, toluene/petroleum ether or acetic acid.) Water-insoluble acids can be partially purified by dissolution in N sodium hydroxide solution and precipitation with dilute mineral acid. If the acid is required to be free from sodium ions, then it is better to dissolve the acid in hot N ammonia, heat to *ca* 80°, adding slightly more than an equal volume of N formic acid and allowing to cool slowly for crystallisation.

The separation and purification of naturally occurring fatty acids, based on distillation, salt solubility and low temperature crystallisation, are described by K.S.Markley (ed), *Fatty Acids*, 2nd edn, part 3, Chap. 20, Interscience, New York. 1964.

Aromatic carboxylic acids can be purified by conversion to their sodium salts, recrystallisation from hot water, and reconversion to the free acids.

(b) Sulphonic: The low solubility of sulphonic acids in organic solvents and their high solubility in water makes necessary a treatment different from that for carboxylic acids. Sulphonic acids are strong, they have the tendency to hydrate, and many of them contain water of crystallisation. The lower-melting and liquid acids can generally be purified with only slight decomposition by fractional distillation, preferably under reduced pressure. A common impurity is sulphuric acid, but this can be removed by recrystallisation from concentrated aqueous solutions. The wet acid can be dried by azeotropic removal of water with toluene, followed by distillation. The higher-melting acids, or acids that melt with decomposition, can be recrystallised from water or, occasionally, from ethanol.

(c) Sulphinic: These acids are less stable, less soluble and less acidic than the corresponding sulphonic acids. The common impurities are the respective sulphonyl chlorides from which they have been prepared, and the thiolsulphonates (neutral) and sulphonic acids into which they decompose. The first two of these can be removed by solvent extraction from an alkaline solution of the acid. On acidification of an alkaline solution, the sulphinic acid crystallises out leaving the sulphonic acid behind. The lower molecular weight members are isolated as their metal (e.g. ferric) salts, but the higher members can be crystallised from water (made slightly acidic), or alcohol.

Acid chlorides. The corresponding acid and hydrogen chloride are the most likely impurities. Usually these can be removed by efficient fractional distillation. Where acid chlorides are not readily hydrolysed (e.g. aryl sulphonyl chlorides) the compound can be freed from contaminants by dissolving in a suitable solvent such as alcohol-free chloroform, dry toluene or petroleum ether and shaking with dilute sodium bicarbonate solution. The organic phase is then washed with water, dried with sodium sulphate or magnesium sulphate, and distilled. This procedure is *hazardous* with readily hydrolysable acid chlorides such as acetyl chloride and benzoyl chloride. Solid acid chlorides are satisfactorily crystallised from toluene, toluene-petroleum ether, petroleum ethers, alcohol-free chloroform/toluene, and, occasionally, from dry ethyl ether. Hydroxylic or basic solvents should be strictly avoided. *All operations should be carried out in a fume cupboard because of the* ***irritant*** *nature of these compounds.*

Alcohols. (a) Monohydric: The common impurities in alcohols are aldehydes or ketones, and water. [*Ethanol* in Chapter 3 is typical.] Aldehydes and ketones can be removed by adding a small amount of sodium metal and refluxing for 2 hours, followed by distillation. Water can be removed in a similar way but it is preferable to use magnesium metal instead of sodium because it forms a more insoluble hydroxide, thereby shifting the equilibrium more completely from metal alkoxide to metal hydroxide. The magnesium should be activated with iodine (or a small amount of methyl iodide), and the water content should be low, otherwise the magnesium will be deactivated.

Acidic materials can be removed by treatment with anhydrous Na_2CO_3, followed by a suitable drying agent, such as calcium hydride, and fractional distillation, using gas chromatography to establish the purity of the product [Ballinger and Long, *JACS* **82** 795 *1960*]. Alternatively, the alcohol can be refluxed with freshly ignited CaO for 4 hours and then fractionally distilled [McCurdy and Laidler, *Canad J Chem* **41** 1867 *1963*].

With higher-boiling alcohols it is advantageous to add some freshly prepared magnesium ethoxide solution (only slightly more than required to remove the water), followed by fractional distillation. Alternatively, in such cases, water can be removed by azeotropic distillation with toluene. Higher-melting alcohols can be purified by crystallisation from methanol or ethanol, toluene/petroleum ether or petroleum ethers. Sublimation in vacuum, molecular distillation and gas chromatography are also useful means of purification. For purification *via* derivatives, see p. 50.

(b) Polyhydric: These alcohols are more soluble in water than are the monohydric ones. Liquids can be freed from water by shaking with type 4A Linde molecular sieves and can safely be distilled only under high vacuum. Carbohydrate alcohols can be crystallised from strong aqueous solution or, preferably, from mixed solvents such as ethanol/petroleum ether or dimethyl formamide/toluene. Crystallisation usually requires seeding and is extremely slow. Further purification can be effected by conversion to the acetyl derivatives which are much less soluble in water and which can readily be recrystallised, e.g. from ethanol. Hydrolysis of the acetyl derivatives, followed by removal of acetate and metal ions by ion-exchange chromatography, gives the purified material. On no account should solutions of carbohydrates be concentrated above 40° because of darkening and formation of *caramel*. Ion exchange, charcoal or cellulose column chromatography has been used for the purification and separation of carbohydrates.

Aldehydes. Common impurities found in aldehydes are the corresponding alcohols, aldols and water from self-condensation, and the corresponding acids formed by autoxidation. Acids can be removed by shaking with aqueous 10% sodium bicarbonate solution. The organic liquid is then washed with water. It is dried with sodium sulphate or magnesium sulphate and then fractionally distilled. Water soluble aldehydes must be dissolved in a suitable solvent such as ethyl ether before being washed in this way. Further purification can be effected *via* the bisulphite derivative (see p. 51) or the Schiff base formed with aniline or benzidine. Solid aldehydes can be dissolved in ethyl ether and purified as above. Alternatively, they can be steam distilled, then sublimed and crystallised from toluene or petroleum ether.

Amides. Amides are stable compounds. The lower-melting members (such as acetamide) can be readily purified by fractional distillation. Most amides are solids which have low solubilities in water. They can be recrystallised from large quantities of water, ethanol, ethanol/ether, aqueous ethanol, chloroform/toluene, chloroform or acetic acid. The likely impurities are the parent acids or the alkyl esters from which they have been made. The former can be removed by thorough washing with aqueous ammonia followed by recrystallisation, whereas elimination of the latter is by trituration or recrystallisation from an organic solvent. Amides can be freed from solvent or water by drying below their melting points. These purifications can also be used for sulphonamides and acid hydrazides.

Amines. The common impurities found in amines are nitro compounds (if prepared by reduction), the corresponding halides (if prepared from them) and the corresponding carbamate salts. Amines are dissolved in aqueous acid, the pH of the solution being at least three units below the pK_a value of the base to ensure almost complete formation of the cation. They are extracted with ethyl ether to remove neutral impurities and to decompose the carbamate salts. The solution is then made strongly alkaline and the amines that separate are extracted into a suitable solvent (ether or toluene) or steam distilled. The latter process removes coloured impurities. Note that chloroform cannot be used as a solvent for primary amines because, in the presence of alkali, poisonous carbylamines are formed. However, chloroform is a useful solvent for the extraction of heterocyclic bases. In this case it has the added advantage that while the extract is being freed from the chloroform most of the moisture is removed with the solvent.
Alternatively, the amine may be dissolved in a suitable solvent (e.g. toluene) and dry HCl gas is passed through the solution to precipitate the amine hydrochloride. This is purified by recrystallisation from a suitable solvent mixture (e.g. ethanol/ethyl ether). The free amine can be regenerated by adding sodium hydroxide and isolated as above.
Liquid amines can be further purified *via* their acetyl or benzoyl derivatives (see p. 51). Solid amines can be recrystallised from water, alcohol, toluene or toluene-petroleum ether. *Care should be taken in handling large quantities of amines because their vapours are **harmful** and they are readily absorbed through the skin.*

Amino acids. Because of their zwitterionic nature, amino acids are soluble in water. Their solubility in organic solvents rises as the fat-soluble portion of the molecule increases. The likeliest impurities are traces of salts, heavy metal ions, proteins and other amino acids. Purification of these is usually easy, by recrystallisation from water or ethanol/water mixtures. The amino acid is dissolved in the boiling solvent, decolorised if necessary by boiling with 1g of acid-washed charcoal/100g amino acid, then filtered hot, chilled, and stood for several hours to crystallise. The crystals are filtered off, washed with ethanol, then ether, and dried.
Amino acids have high melting or decomposition points and are best examined for purity by paper or thin layer chromatography. The spots are developed with ninhydrin (see Lederer and Lederer, p.44). Customary methods for the purification of small quantities of amino acids obtained from natural sources (i.e. 1-5g) are ion-exchange chromatography (see p. 20) or countercurrent distribution (see p. 28). For general treatment of amino acids see Greenstein and Winitz [*The Amino Acids*, Vols 1-3, J.Wiley & Sons, New York 1961].
A useful source of details such as likely impurities, stability and tests for homogeneity of amino acids is *Specifications and Criteria for Biochemical Compounds*, 3rd edn, 1972, National Academy of Sciences, USA].

Anhydrides. The corresponding acids, resulting from hydrolysis, are the most likely impurities. Distillation from phosphorus pentoxide, followed by fractional distillation, is usually satisfactory. With high boiling or solid anhydrides, another method involves refluxing for 0.5-1 hour with acetic anhydride, followed by fractional distillation. Acetic acid distils first, then acetic anhydride and finally the desired anhydride. Where the anhydride is a solid, removal of acetic acid and acetic anhydride at atmospheric pressure is followed by heating under vacuum. The solid anhydride is then either crystallised as for acid chlorides or (in some cases) sublimed in a vacuum. A preliminary purification when large quantities of acid are present in a solid anhydride (such as phthalic anhydride) can sometimes be

achieved by preferential solvent extraction of the (usually) more soluble anhydride from the acid (e.g. with chloroform in the case of phthalic anhydride). ***All operations with liquid anhydrides should be carried out in a fume cupboard because of their*** **LACHRYMATORY** ***properties.***

Carotenoids. These usually are decomposed by light, air and solvents, so that degradation products are probable impurities. Chromatography and adsorption spectra permit the ready detection of coloured impurities, and separations are possible using solvent distribution, chromatography or crystallisation. Thus, in partition between immiscible solvents, xanthophyll remains in 90% methanol while carotenes pass into the petroleum ether phase. For small amounts of material, thin-layer or paper chromatography may be used, while column chromatography is suitable for larger amounts. Colourless impurities may be detected by IR, NMR or mass spectrometry. The more common separation procedures are described by P.Karrer and E.Jucker in *Carotenoids*, E.A.Braude (translator), Elsevier, NY, 1950.
Purity can be assayed by chromatography (on thin-layer plates, Kieselguhr paper or columns), by UV or NMR procedures.

Esters. The most common impurities are the corresponding acid and hydroxy compound (i.e. alcohol or phenol), and water. A liquid ester from a carboxylic acid is washed with 2N sodium carbonate or sodium hydroxide to remove acid material, then shaken with calcium chloride to remove ethyl or methyl alcohols (if it is a methyl or ethyl ester). It is dried with potassium carbonate or magnesium sulphate, and distilled. Fractional distillation then removes residual traces of hydroxy compounds. This method does not apply to esters of inorganic acids (e.g. dimethyl sulphate) which are more readily hydrolysed in aqueous solution when heat is generated in the neutralisation of the excess acid. In such cases, several fractional distillations, preferably under vacuum, are usually sufficient.
Solid esters are easily crystallisable materials. It is important to note that esters of alcohols must be recrystallised either from non-hydroxylic solvents (e.g. toluene) or from the alcohol from which the ester is derived. Thus methyl esters should be crystallised from methanol or methanol/toluene, but not from ethanol, *n*-butanol or other alcohols, in order to avoid alcohol exchange and contamination of the ester with a second ester. Useful solvents for crystallisation are the corresponding alcohols or aqueous alcohols, toluene, toluene/petroleum ether, and chloroform (ethanol-free)/toluene. Carboxylic acid esters derived from phenols are more difficult to hydrolyse and exchange, hence any alcoholic solvent can be used freely. Sulphonic acid esters of phenols are even more resistant to hydrolysis: they can safely be crystallised not only from the above solvents but also from acetic acid, aqueous acetic acid or boiling *n*-butanol.
Fully esterified phosphoric acid and phosphonic acids differ only in detail from the above mentioned esters. Their major contaminants are alcohols or phenols, phosphoric or phosphonic acids (from hydrolysis), and (occasionally) basic material, such as pyridine, which is used in their manufacture. Water-insoluble esters are washed thoroughly and successively with dilute acid (e.g. 0.2N sulphuric acid), water, 0.2N sodium hydroxide and water. After drying with calcium chloride they are fractionally distilled. Water-soluble esters should first be dissolved in a suitable organic solvent and, in the washing process, water should be replaced by saturated aqueous sodium chloride. Some esters (e.g. phosphate and phosphonate esters) can be further purified through their uranyl adducts (see p. 53). Traces of water or hydroxy compounds can be removed by percolation through, or shaking with, activated alumina (about 100g/L of liquid solution), followed by filtration and fractional distillation in a vacuum. For high molecular weight esters (which cannot be distilled without some decomposition) it is advisable to carry out distillation at as low a pressure as possible. Solid esters can be crystallised from toluene or petroleum ether. Alcohols can be used for recrystallising phosphoric or phosphonic esters of phenols.

Ethers. The purification of ethyl ether (see Chapter 3) is typical of liquid ethers. The most common contaminants are the alcohols or hydroxy compounds from which the ethers are prepared, their oxidation products (e.g. aldehydes), peroxides and water. Peroxides, aldehydes and alcohols can be removed by shaking with alkaline potassium permanganate solution for several hours, followed by washing with water, concentrated sulphuric acid, then water. After drying with calcium chloride, the ether is distilled. It is then dried with sodium or with lithium aluminium hydride, redistilled and given a final fractional distillation. The drying process should be repeated if necessary.
Alternatively, methods for removing peroxides include leaving the ether to stand in contact with iron filings or copper powder, shaking with a solution of ferrous sulphate acidified with sulphuric acid, shaking with a copper-zinc couple, passage through a column of activated alumina, and refluxing with phenothiazine. Cerium(III) hydroxide has also been used.
A simple test for ether peroxides is to add 10ml of the ether to a stoppered cylinder containing 1ml of freshly prepared 10% solution of potassium iodide containing a drop of starch indicator. No colour should develop during one minute. Alternatively, a 1% solution of ferrous ammonium sulphate, 0.1M in sulphuric acid and 0.01M in potassium thiocyanate should not increase appreciably in red colour when shaken with two volumes of the ether.
As a safety precaution against **EXPLOSION** (in case the purification has been insufficiently thorough) at least a quarter of the total volume of ether should remain in the distilling flask when the distillation is discontinued. To minimize peroxide formation, ethers should be stored in dark bottles and, if they are liquids, they should be left in contact with type 4A Linde molecular sieves, in a cold place, over sodium amalgam. The rate of formation of peroxides depends on storage conditions and is accelerated by heat, light, air and moisture. The formation of peroxides is inhibited in the presence of diphenylamine, di-*tert*-butylphenol, or other antioxidant as stabilizer.

Ethers that are solids (e.g. phenyl ethers) can be steam distilled from an alkaline solution which will hold back any phenolic impurity. After the distillate is made alkaline with sodium carbonate, the insoluble ether is collected either by extraction (e.g. with chloroform, ethyl ether or toluene) or by filtration. It is then crystallised from alcohols, alcohol/petroleum ether, petroleum ether, toluene or mixtures of these solvents, sublimed in a vacuum and recrystallised.

Halides. Aliphatic halides are likely to be contaminated with halogen acids and the alcohols from which they have been prepared, whereas in aromatic halides the impurities are usually aromatic hydrocarbons, amines or phenols. In both groups the halogen atom is less reactive than it is in acid chlorides. Purification is by shaking with concentrated hydrochloric acid, followed by washing successively with water, 5% sodium carbonate or bicarbonate, and water. After drying with calcium chloride, the halide is distilled and then fractionally distilled using an efficient column. For a solid halide the above purification is carried out by dissolving it in a suitable solvent such as toluene. Solid halides can also be purified by chromatography using an alumina column and eluting with toluene or petroleum ether. They can be crystallised from toluene, petroleum ethers, toluene/petroleum ether or toluene/chloroform/petroleum ether. Care should be taken when handling organic halogen compounds because of their **TOXICITY.**
Liquid aliphatic halides are obtained alcohol-free by distillation from phosphorus pentoxide. They are stored in dark bottles to prevent oxidation and, in some cases, the formation of phosgene.
A general method for purifying *chlorohydrocarbons* uses repeated shaking with concentrated sulphuric acid until no further colour develops in the acid, then washing with a solution of sodium bicarbonate, followed by water. After drying with calcium chloride, the chlorohydrocarbon is fractionally redistilled to constant boiling point.

Hydrocarbons. Gaseous hydrocarbons are best freed from water and gaseous impurities by passage through suitable adsorbents and (if olefinic material is to be removed) oxidants such as alkaline potassium permanganate solution, followed by fractional cooling (see p. 36 for cooling baths) and fractional distillation at low temperature. To effect these purifications and also to store the gaseous sample, a vacuum line is necessary.
Impurities in hydrocarbons can be characterised and evaluated by gas chromatography and mass spectrometry. The total amount of impurities present can be estimated from the thermometric freezing curve.
Liquid aliphatic hydrocarbons are freed from aromatic impurities by shaking with concentrated sulphuric acid whereby the aromatic compounds are sulphonated. Shaking is carried out until the sulphuric acid layer remains colourless for several hours. The hydrocarbon is then freed from the sulphuric acid and the sulphonic acids by separating the two phases and washing the organic layer successively with water, 2N sodium hydroxide, and water. It is dried with $CaCl_2$ or Na_2SO_4, and then distilled. The distillate is dried with sodium wire, P_2O_5, or metallic hydrides, or passage through a dry silica gel column, or preferably, and more safely, with molecular sieves (see p. 28) before being finally fractionally distilled through an efficient column. If the hydrocarbon is contaminated with olefinic impurities, shaking with aqueous alkaline permanganate is necessary prior to the above purification. Alicyclic and paraffinic hydrocarbons can be freed from water, non-hydrocarbon and aromatic impurities by passage through a silica gel column before the final fractional distillation. This may also remove isomers. (For the use of chromatographic methods to separate mixtures of aromatic, paraffinic and alicyclic hydrocarbons see references on pp. 44 and 45 under *Chromatography, Gas Chromatography and High Perforamance Liquid Chromatography*). Another method of removing branched-chain and unsaturated hydrocarbons from straight-chain hydrocarbons depends on the much faster reaction of the former with chlorosulphonic acid.
Isomeric materials which have closely similar physical properties can be serious contaminants in hydrocarbons. With aromatic hydrocarbons, e.g. xylenes and alkyl benzenes, advantage is taken of differences in ease of sulphonation. If the required compound is sulphonated more readily, the sulphonic acid is isolated, crystallised (e.g. from water), and decomposed by passing superheated steam through the flask containing the acid. The sulphonic acid undergoes hydrolysis and the liberated hydrocarbon distils with the steam. It is separated from the distillate, dried, distilled and then fractionally distilled. For small quantities (10-100mg), vapour phase chromatography is the most satisfactory method for obtaining a pure sample (for column materials for packings see p. 25).
Azeotropic distillation with methanol or 2-ethoxyethanol has been used to obtain highly purified saturated hydrocarbons and aromatic hydrocarbons such as xylenes and isopropylbenzenes.
Carbonyl-containing impurities can be removed from hydrocarbons (and other oxygen-lacking solvents such as $CHCl_3$ and CCl_4) by passage through a column of Celite 545 (100g) mixed with concentrated sulphuric acid (60ml). After first adding some solvent and about 10g of granular Na_2SO_4, the column is packed with the mixture and a final 7-8cm of Na_2SO_4 is added at the top [Hornstein and Crowe, *AC* **34** 1037 *1962*]. Alternatively, Celite impregnated with 2,4-dinitrophenylhydrazine can be used.
With solid hydrocarbons such as naphthalene, preliminary purification by sublimation in vacuum (or high vacuum if the substance is high melting), is followed by zone refining and finally by chromatography (e.g. on alumina) using low-boiling liquid hydrocarbon eluents. These solids can be recrystallised from alcohols, alcohol/petroleum ether or from liquid hydrocarbons (e.g. toluene) and dried below their melting points. Aromatic hydrocarbons that have been purified by zone melting include anthracene, biphenyl, fluoranthrene, naphthalene, perylene, phenanthrene, pyrene and terphenyl, among others.
Olefinic hydrocarbons have a very strong tendency to polymerise and commercially available materials are generally stabilized, e.g. with hydroquinone. When distilling compounds such as vinylpyridine or styrene, the stabilizer remains behind and the purified olefinic material is more prone to polymerization. The most common impurities are

higher-boiling dimeric or polymeric compounds. Vacuum distillation in a nitrogen atmosphere not only separates monomeric from polymeric materials but in some cases also depolymerizes the impurities. The distillation flask should be charged with a polymerization inhibitor and the purified material should be used immediately or stored in the dark and mixed with a small amount of stabilizer (e.g. 0.1% of hydroquinone).

Imides. Imides (e.g. phthalimide) can be purified by conversion to their potassium salts by reaction in ethanol with ethanolic potassium hydroxide. The imides are regenerated when the salts are hydrolysed with dilute acid. Like amides, imides readily crystallise from alcohols and, in some cases (e.g. quinolinic imide), from glacial acetic acid.

Imino compounds. These substances contain the -C=NH group and, because they are strong, unstable bases, they are kept as their more stable salts, such as the hydrochlorides. (The free base usually hydrolyses to the corresponding oxo compound and ammonia.) Like amine hydrochlorides, the salts are purified by solution in alcohol containing a few drops of hydrochloric acid. After treatment with charcoal, and filtering, dry ethyl ether (or petroleum ether if ethanol is used) is added until crystallisation sets in. The salts are dried and kept in a vacuum desiccator.

Ketones. Ketones are more stable to oxidation than aldehydes and can be purified from oxidisable impurities by refluxing with potassium permanganate until the colour persists, followed by shaking with sodium carbonate (to remove acidic impurities) and distilling. Traces of water can be removed with type 4A Linde molecular sieves. Ketones which are solids can be purified by crystallisation from alcohol, toluene, or petroleum ether, and are usually sufficiently volatile for sublimation in vacuum. Ketones can be further purified *via* their bisulphite, semicarbazone or oxime derivatives (see p. 51). The bisulphite addition compounds are formed only by aldehydes and methyl ketones but they are readily hydrolysed in dilute acid or alkali.

Macromolecules. See Chapter 5.

Nitriles. *All purifications should be carried out in an efficient fume cupboard because of the* **TOXIC** *nature of these compounds.*
Nitriles are usually prepared either by reacting the corresponding halide or diazonium salts with a cyanide salt or by dehydrating an amide. Hence, possible contaminants are the respective halide or alcohol (from hydrolysis), phenolic compounds, amines or amides. Small quantities of phenols can be removed by chromatography on alumina. More commonly, purification of liquid nitriles or solutions of solid nitriles in a solvent such as ethyl ether is by shaking with dilute aqueous sodium hydroxide, followed by washing successively with water, dilute acid and water. After drying with sodium sulphate, the solvent is distilled off. Liquid nitriles are best distilled from a small amount of P_2O_5 which, besides removing water, dehydrates any amide to the nitrile. About one fifth of the nitrile should remain in the distilling flask at the end of the distillation (*the residue may contain some inorganic cyanide*). This purification also removes alcohols and phenols. Solid nitriles can be recrystallised from ethanol, toluene or petroleum ether, or a mixture of these solvents. They can also be sublimed under vacuum. Preliminary purification by steam distillation is usually possible.
Strong alkali or heating with dilute acids may lead to hydrolysis of the nitrile, and should be avoided.

Nitro compounds. Aliphatic nitro compounds are acidic. They are freed from alcohols or alkyl halides by standing for a day with concentrated sulphuric acid, then washed with water, dried with magnesium sulphate followed by calcium sulphate and distilled. The principal impurities are isomeric or homologous nitro compounds. In cases where the nitro compound was originally prepared by vapour phase nitration of the aliphatic hydrocarbon, fractional distillation should separate the nitro compound from the corresponding hydrocarbon. Fractional crystallisation is more effective than fractional distillation if the melting point of the compound is not too low.
The impurities present in aromatic nitro compounds depend on the aromatic portion of the molecule. Thus, benzene, phenols or anilines are probable impurities in nitrobenzene, nitrophenols and nitroanilines, respectively. Purification should be carried out accordingly. Isomeric compounds are likely to remain as impurities after the preliminary purifications to remove basic and acidic contaminants. For example, *o*-nitrophenol may be found in samples of *p*-nitrophenol. Usually, the *o*-nitro compounds are more steam volatile than the *p*-nitro isomers, and can be separated in this way. Polynitro impurities in mononitro compounds can be readily removed because of their relatively lower solubilities in solvents. With acidic or basic nitro compounds which cannot be separated in the above manner, advantage may be taken of their differences in pK_a values. The compounds can thus be purified by preliminary extractions with several sets of aqueous buffers of known pH (see for example Table 19, p. 43) from a solution of the substance in a suitable solvent such as ethyl ether. This method is more satisfactory and less laborious the larger the difference between the pK_a value of the impurity and the desired compound. Heterocyclic nitro compounds require similar treatment to the nitroanilines. Neutral nitro compounds can be steam distilled.

Nucleic acids. See Chapter 5.

Phenols. Because phenols are weak acids, they can be freed from neutral impurities by dissolution in aqueous N sodium hydroxide and extraction with a solvent such as ethyl ether, or by steam distillation to remove the non-acidic

material. The phenol is recovered by acidification of the aqueous phase with 20% sulphuric acid, and either extracted with ether or steam distilled. In the second case the phenol is extracted from the steam distillate after saturating it with sodium chloride. A solvent is necessary when large quantities of liquid phenols are purified. The phenol is fractionated by distillation under reduced pressure, preferably in an atmosphere of nitrogen to minimize oxidation. Solid phenols can be crystallised from toluene, petroleum ether or a mixture of these solvents, and can be sublimed under vacuum. Purification can also be effected by fractional crystallisation or zone refining. For further purification of phenols *via* their acetyl or benzoyl derivatives, see p. 53.

Polypeptides and proteins . See Chapter 5.

Quinones. These are neutral compounds which are usually coloured. They can be separated from acidic or basic impurities by extraction of their solutions in organic solvents with aqueous basic or acidic solutions, respectively. Their colour is a useful property in their purification by chromatography through an alumina column with, e.g. toluene as eluent. They are volatile enough for vacuum sublimation, although with high-melting quinones a very high vacuum is necessary. *p*-Quinones are stable compounds and can be recrystallised from water, ethanol, aqueous ethanol, toluene, petroleum ether or glacial acetic acid. *o*-Quinones, on the other hand, are readily oxidised. They should be handled in an inert atmosphere, preferably in the absence of light.

Salts (organic). (a) With metal ions: Water-soluble salts are best purified by preparing a concentrated aqueous solution to which, after decolorising with charcoal and filtering, ethanol or acetone is added so that the salts crystallise. They are collected, washed with aqueous ethanol or aqueous acetone, and dried. In some cases, water-soluble salts can be recystallised satisfactorily from alcohols. Water-insoluble salts are purified by Soxhlet extraction, first with organic solvents and then with water, to remove soluble contaminants. The purified salt is recovered from the thimble.

(b) With organic ions: Organic salts (e.g. trimethylammonium benzoate) are usually purified by recrystallisation from polar solvents (e.g. water, ethanol or dimethyl formamide). If the salt is too soluble in a polar solvent, its concentrated solution should be treated dropwise with a miscible nonpolar solvent (see p. 14) until crystallisation begins.

(c) Sodium alkane disulphonates: Purified from sulphites by boiling with aq HBr. Purified from sulphates by adding $BaBr_2$. Sodium alkane disulphonates are finally pptd by addition of MeOH. [Pethybridge and Taba *JCSFT 1* **78** 1331 *1982*].

Sulphur compounds. (a) Disulphides can be purified by extracting acidic and basic impurities with aqueous base or acid, respectively. However, they are somewhat sensitive to strong alkali which slowly cleaves the disulphide bond. The lower-melting members can be fractionally distilled under vacuum. The high members can be recrystallised from alcohol, toluene or glacial acetic acid.

(b) Sulphones are neutral and extremely stable compounds that can be distilled without decomposition. They are freed from acidic and basic impurities in the same way as disulphides. The low molecular weight members are quite soluble in water but the higher members can be recrystallised from water, ethanol, aqueous ethanol or glacial acetic acid.

(c) Sulphoxides are odourless, rather unstable compounds, and should be distilled under vacuum in an inert atmosphere. They are water-soluble but can be extracted from aqueous solution with a solvent such as ethyl ether.

(d) Thioethers are neutral stable compounds that can be freed from acidic and basic impurities as described for disulphides. They can be recrystallised from organic solvents and distil without decomposition.

(e) Thiols are stronger acids than the corresponding hydroxy compounds but can be purified in a similar manner. However, care must be exercised in handling thiols to avoid their oxidation to disulphides. For this reason, purification is best carried out in an inert atmosphere in the absence of oxidising agents. Similarly, thiols should be stored out of contact with air. They can be distilled without change, and the higher-melting thiols (which are usually more stable) can be crystallised, e.g. from water or dilute alcohol. They oxidise readily in alkaline solution but can be separated from the disulphide which is insoluble in this medium. They should be stored in the dark below 0°. *All operations with thiols should be carried out in an efficient fume cupboard because of their unpleasant odour and their* **TOXICITY**.

(f) Thiolsulphonates (disulphoxides) are neutral and are somewhat light-sensitive compounds. Their most common impurities are sulphonyl chlorides (neutral) or the sulphinic acid or disulphide from which they are usually derived. The first can be removed by partial freezing or crystallisation, the second by shaking with dilute

alkali, and the third by recrystallisation because of the higher solubility of the disulphide in solvents. Thiolsulphonates decompose slowly in dilute, or rapidly in strong, alkali to form disulphides and sulphonic acids. Thiolsulphonates also decompose on distillation but they can be steam distilled. The solid members can be recrystallised from water, alcohols or glacial acetic acid.

BIBLIOGRAPHY

Characterization of Organic and Inorganic Compounds

G.Bauer, *Handbook of Preparative Inorganic Chemistry*, 2nd edn, Vol 1 (1963) and vol 2 (1965), Academic Press, NY.

CRC - Handbook of Chemistry and Physics, 76th edn, CRC Press Inc., Boca Raton, Florida, 1995-1996.

N.D.Cheronis and J.B.Entrikin, *Identification of Organic Compounds*, Interscience, NY, 1963.

F Feigl and V. Auger, *Spot Tests in Organic Analysis*, 7th edn, Elsevier, Amsterdam, 1989.

M.Fieser and L.Fieser, *Reagents for Organic Synthesis*, J.Wiley & Sons, Inc., NY, vol **1** *1967* to vol **15**, 1990.

W.J.Hickinbottom, *Reactions of Organic Compounds*, Longmans, London, 3rd edn, 1958.

S.M.McElvain, *The Characterisation of Organic Compounds*, Macmillan, NY, 2nd edn, 1958.

Inorganic Synthesis, Magraw-Hill Book Co, Inc., NY, vol I (1939) to vol XVII (1977), Wiley Interscience Publ., NY, vol XVIII (1978) to vol **30** 1995.

R.B.King (ed. in chief), *Encyclopedia of Inorganic Chemistry*, (8 volumes), J.Wiley & Sons Ltd., NY, 1994.

Organic Synthesis, J.Wiley & Sons Ltd., NY, col vol I (1941) to vol 73 (1996).

D.R.Lide and G.W.A.Milne, *Handbook of Data on Common Organic Compounds*, CRC Press, Boca Raton, Florida, 1995.

L.A.Paquette (ed. in chief), *Encyclopedia of Reagents for Organic Synthesis*, (8 volumes), J.Wiley & Sons, NY, 1995.

R.L.Shriner and R.C.Fuson, *The Systematic Identification of Organic Compounds*, J.Wiley & Sons, NY, 3rd edn, 1948.

B.S.Furniss, A.J.Hannaford, V.Rogers, P.W.G.Smith and A.R.Tatchell, *Vogel's Textbook of Practical Organic Chemistry*, Longmans, London, 4th edn, 1978.

D.Lin-Vien, N.B.Colthup, W.G.Fateley and J.G.Grasselli, *Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*, Academic Press, NY, 1991.

R.C.Weast, *Physical Constants of Inorganic Compounds*, in *CRC Handbook of Chemistry and Physics*, CRC Press, Cleveland, Ohio, 58th edn, 1978.

Metal Hydrides

G.Bambakidis, *Metal Hydrides*, Plenum Press, NY, 1981.

N.G.Gaylord, *Reductions with Complex Metal Hydrides*, Interscience, NY, 1956.

Sodium Borohydride and Potassium Borohydride: A Manual of Techniques, Metal Hydrides, Beverly, Massachusettes, 1958.

Spectroscopy

F.W.McLafferty and D.B.Stauffer, *The Wiley/NBS Registry of Mass Spectral Data*, (7 volumes), J.Wiley & Sons, NY, 1989.

J.A.McClosky, Mass Spectrometry *Methods in Enzymology* **193** *1990*.

C.J.Pouchet, *The Aldrich Library of NMR Spectra*, 2nd edn, (2 volumes), Aldrich Chemical Co. Inc., 1983.

C.J.Pouchet and J.Behnke, *The Aldrich Library of ^{13}C and ^{1}H FT-NMR Spectra*, (3 volumes), Aldrich Chemical Co. Inc., 1993.

C.J.Pouchet, *The Aldrich Library of Infrared Spectra*, 3rd edn, (3 volumes), Aldrich Chemical Co. Inc., 1981.

Trace Metal Analysis

F.Feigl and V.Anger, *Spot Tests in Inorganic Analysis*, Elsevier, Amsterdam, 1972.
A.Varma, *Handbook of Atomic Absorption Analysis*, (2 volumes), CRC Press, Boca Raton, Florida, 1984
B.Welz (translated by C.Skegg), *Atomic Absorption Spectrometry*, VCH Publ., Weinheim, 1985.
N.Zief and J.W.Mitchell, *Contamination Control in Trace Analysis*, Wiley, NY, 1979.